

Plaque Reduction Neutralization Test (PRNT)

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An Antibody from Single Human VH-rearranging Mouse Neutralizes All SARS-CoV-2 Variants Through BA.5 by Inhibiting Membrane Fusion

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Detailed protocol

SARS-CoV-2 Plaque Reduction Neutralization Tests (PRNT) were performed in the Duke Regional Biocontainment Laboratory BSL3 (Durham, NC) as previously described with virus-specific modifications (62). Vero E6 cells (ATCC CRL-1586) were maintained in growth medium (MEM +Earl's Salts +L-Glutamine Gibco 11095, 100 U/mL Penicillin and 100ug/mL Streptomycin Gibco 15140, 1 mM sodium pyruvate Gibco 11360, 1X non-essential amino acids Gibco 11140, and 10% fetal bovine serum Gemini 100-106). One day before the assay 1.4e5 cells were placed in each well of a 12 well tissue culture plate. On the day of the assay, antibodies were diluted in virus diluent (MEM +Earl's Salts +L-Glutamine Gibco 11095, 100 U/mL Penicillin and 100ug/mL Streptomycin Gibco 15140, 1 mM sodium pyruvate Gibco 11360, 1X non-essential amino acids Gibco 11140, and 2% fetal bovine serum Gemini 100-106). Samples were diluted tenfold, and the range of concentrations tested was from 10 µg/mL – 0.0001 µg/mL.

Viruses were obtained from BEI Resources: USA-WA1/2020 (NR-52281), USA/CA_CDC_5574/2020 (NR-54011), South Africa/KRISP-EC-K005321/2020 (NR-54008), Japan/TY7/503/2021 (NR-54982), and USA/PHC658/2021 (NR-55611). Each virus was propagated on Vero E6 cells and frozen in single use aliquots. Antibody samples were mixed with an equal volume of virus diluent containing 50 PFU. This antibody – virus mixture was incubated at 37°C for 1 hour. Each plate was washed, and 0.2 mL of each antibody – virus mixture was added to the appropriate well. Samples and cells were incubated at 37°C for 1 hour. At the end of the incubation, 1 mL of a viscous overlay (1:1 2X Virus Diluent and 1.2% methylcellulose) was added to each well. Plates were incubated at 37°C for 96 hours.

After incubation, contents of each well were aspirated, and each well was flooded with 10% neutral buffered formalin for 30 minutes. Plates were then treated with 0.1% Crystal Violet for 15 minutes, and excess crystal violet was washed with tap water. At all times during this assay, care was taken to not wound or dislodge the monolayer.

After fixation, staining and washing, plates were dried and plaques from each dilution of each sample were counted. Percent inhibition was calculated from each plaque count and used to determine IC_{50/90} values with non-linear regression analysis tools from GraphPad Prism. In each assay, samples were measured in duplicate alongside established positive (Clone D001; SINO, CAT# 40150-D001) and negative controls (known non-neutralizing antibodies).

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Luo, S. , Kirchhausen, T. , Chen, B. , Haynes, B. and Alt, F. (2022). Plaque Reduction Neutralization Test (PRNT). Bio-protocol Preprint. bio-protocol.org/prep1946.
2. Luo, S., Zhang, J., Kreutzberger, A. J., Eaton, A., Edwards, R. J., Jing, C., Dai, H., Sempowski, G. D., Cronin, K., Parks, R., Ye, A. Y., Mansouri, K., Barr, M., Pishesha, N., Williams, A. C., Vieira Francisco, L., Saminathan, A., Peng, H., Batra, H., Bellusci, L., Khurana, S., Alam, S. M., Montefiori, D. C., Saunders, K. O., Tian, M., Ploegh, H., Kirchhausen, T., Chen, B., Haynes, B. F. and Alt, F. W.(2022). An Antibody from Single Human VH-rearranging Mouse Neutralizes All SARS-CoV-2 Variants Through BA.5 by Inhibiting Membrane Fusion. Science Immunology. DOI: [10.1126/sciimmunol.add5446](https://doi.org/10.1126/sciimmunol.add5446)

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